

This listing of claims will replace all prior versions, and listing, of claims in the application.

LISTING OF CLAIMS:

1. (previously presented) A method for detecting a target nucleic acid in a nucleic acid containing sample comprising:

(a) contacting the nucleic acid containing sample with a circular oligonucleotide probe under conditions that allow hybridization between complementary sequences in the target nucleic acid and the circular oligonucleotide probe;

(b) adding at least one forward primer comprising a sequence complementary to a portion of the circular oligonucleotide probe, under conditions where the forward primer is extended around the circular oligonucleotide probe for multiple revolutions to form a single-stranded DNA molecule comprising repeating units complementary to the sequence of the circular probe;

(c) adding at least one oligonucleotide primer pair comprising a first primer and a second primer, wherein

(i) the first primer of the pair comprises (A) a first sequence on its 3' end that is substantially identical to a portion of the circular oligonucleotide probe, (B) a second sequence that is complementary to the second primer of the pair, and (C) a signal generating moiety selected from the group consisting of a fluorescent agent and a chemiluminescent agent;

- (ii) the second primer of the pair comprises (A) a sequence that is complementary to the first primer and (B) a moiety capable of quenching, masking or inhibiting the activity of the signal generating moiety when located adjacent to, or in close proximity to the signal generating moiety; and
- (iii) when the first primer and the second primer are bound to one another, a signal generated by the signal generating moiety is inhibited;
- (d) adding at least one reverse primer comprising a sequence that is substantially identical to a portion of the circular oligonucleotide probe and wherein the forward primer, reverse primer, and the first primer and the second primer of the oligonucleotide primer pair are not identical;
- (e) adding a DNA polymerase having strand displacement activity and lacking 3' to 5' exonuclease activity; and
- (f) amplifying the circular oligonucleotide probe using ramification-extension amplification method (RAM) thus producing an amplification product comprising a sequence that is substantially identical to a sequence in the circular probe, and separating the signal generating moiety selected from the group consisting of a fluorescent agent and a chemiluminescent agent from the quenching, masking or inhibitory moiety by the action of the DNA polymerase having strand displacement activity and lacking 3' to 5' exonuclease activity during the amplification method, wherein detection of the signal indicates the presence of the target nucleic acid in the nucleic acid containing sample.

2. (previously presented) A method for detecting a target nucleic acid in a nucleic acid containing sample comprising:

(a) contacting the target nucleic acid containing sample with a linear oligonucleotide probe comprising 3' and 5' regions complementary to adjacent sequences in the target nucleic acid under conditions that allow hybridization between complementary sequences in the target nucleic acid and the linear oligonucleotide probe, whereupon binding to the target nucleic acid, the 3' and 5' ends of the linear oligonucleotide probe are adjacent to each other such that ligation of the 3' and 5' ends of the linear oligonucleotide probe forms the circular oligonucleotide probe, ligating the 3' and 5' ends of the linear oligonucleotide probe, and forming a circular oligonucleotide probe when the target nucleic acid is in the nucleic acid containing sample;

(b) adding at least one forward primer comprising a sequence complementary to a portion of the circular oligonucleotide probe, under conditions where the forward primer is extended around the circular oligonucleotide probe for multiple revolutions to form a single-stranded DNA molecule repeating units complementary to the sequence of the circular oligonucleotide probe;

(c) adding at least one oligonucleotide primer pair comprising a first primer and a second primer of the second primer pair, wherein

(i) the first primer of the pair comprises (A) a first sequence on its 3' end that is substantially identical to a portion of the circular oligonucleotide probe, (B) a second sequence that is complementary to the second primer of the pair, and (C) a

signal generating moiety selected from the group consisting of a fluorescent agent and a chemiluminescent agent;

(ii) the second primer of the pair comprises (A) a sequence that is complementary to the first primer and (B) a moiety capable of quenching, masking or inhibiting the activity of the signal generating moiety when located adjacent to, or in close proximity to the signal generating moiety;

(iii) when the first primer and the second primer are bound to one another, a signal generated by the signal generating moiety is inhibited;

(d) adding at least one reverse primer comprising a sequence that is substantially identical to a portion of the circular oligonucleotide probe and wherein the forward primer, reverse primer, and the first primer and the second primer of the oligonucleotide primer pair are not identical;

(e) adding a DNA polymerase having strand displacement activity and lacking 3' to 5' exonuclease activity; and

(f) amplifying the circular oligonucleotide probe using ramification-extension amplification method (RAM) thus producing an amplification product comprising a sequence that is substantially identical to a sequence in the circular probe, and separating the signal generating moiety from the group consisting of a fluorescent agent and a chemiluminescent agent from the quenching, masking or inhibitory moiety by the action of the DNA polymerase having strand displacement activity and lacking 3' to 5'

exonuclease activity during the amplification method, wherein detection of the signal indicates the presence of the target nucleic acid in the nucleic acid containing sample.

3. – 22. (Cancelled)

23. (Currently Amended) A method for detecting a target nucleic acid in a nucleic acid containing sample comprising:

(a) contacting the nucleic acid containing sample with a circular oligonucleotide probe under conditions that allow hybridization between complementary sequences in the target nucleic acid and the circular oligonucleotide probe;

(b) adding at least one multiple oligonucleotide primer complex comprising a first primer, a second primer and a third primer, under conditions where the multiple oligonucleotide primer complex is extended around the circular oligonucleotide probe for multiple revolutions to form a single-stranded DNA molecule comprising repeating units complementary to the sequence of the circular oligonucleotide probe, wherein

(i) the first primer of the multiple oligonucleotide primer complex comprises (A) a first sequence on its 3' end that is complementary to a portion of the circular oligonucleotide probe, (B) a second sequence that is complementary to the second primer of the multiple oligonucleotide primer complex, and (C) a third sequence that is complementary to the third primer of the multiple oligonucleotide primer complex;

(ii) the second primer of the multiple oligonucleotide primer complex comprises (A) a sequence that is complementary to the second sequence of the first primer of

the multiple oligonucleotide primer complex and (B) a signal generating moiety selected from the group consisting of a fluorescent agent and a chemiluminescent agent;

(iii) the third primer of the multiple oligonucleotide primer complex comprises (A) a sequence that is complementary to the third sequence of the first primer of the multiple oligonucleotide primer complex and ~~(b)~~ (B) a moiety capable of quenching, masking or inhibiting the activity of the signal generating moiety when located adjacent to, or in close proximity to the signal generating moiety; and

(iv) when the first, second and third primers of the multiple oligonucleotide primer complex are bound to one another, a signal generated by the signal generating moiety is inhibited;

(c) adding at least one reverse primer comprising a sequence that is substantially identical to a portion of the circular oligonucleotide probe;

(d) adding a DNA polymerase having strand displacement activity and lacking 3' to 5' exonuclease activity; and

(e) amplifying the circular oligonucleotide probe using ramification-extension amplification method (RAM) thus producing an amplification product comprising a sequence that is substantially identical to a sequence in the circular oligonucleotide probe, and separating the signal generating moiety from the quenching, masking or inhibitory moiety to generate a signal by the action of the DNA polymerase having strand

displacement activity and lacking 3' to 5' exonuclease activity during the amplification method, wherein detection of the signal indicates the presence of the target nucleic acid in the nucleic acid containing sample.

24. (Currently Amended) A method for detecting a target nucleic acid in a nucleic acid containing sample comprising:

(a) contacting the target nucleic acid containing sample with a linear oligonucleotide probe comprising 3' and 5' regions complementary to adjacent sequences in the target nucleic acid under conditions that allow hybridization between complementary sequences in the target nucleic acid and the linear oligonucleotide probe, whereupon binding to the target nucleic acid, the 3' and 5' ends of the linear oligonucleotide probe are adjacent to each other such that ligation of the 3' and 5' ends of the linear oligonucleotide probe ~~form~~ forms the circular oligonucleotide probe, ligating the 3' and 5' ends of a the linear oligonucleotide probe, and forming a circular oligonucleotide probe when the target nucleic acid is in the nucleic acid containing sample;

(b) adding at least one multiple oligonucleotide primer complex comprising a first primer, a second primer and a third primer, under conditions where the multiple oligonucleotide primer complex is extended around the circular oligonucleotide probe for multiple revolutions to form a single-stranded DNA molecule comprising repeating units complementary to the sequence of the circular oligonucleotide probe, wherein

(i) the first primer of the multiple oligonucleotide primer complex comprises (A) a first sequence on its 3' end that is complementary to a portion of the circular oligonucleotide probe, (B) a second sequence that is complementary to the second

primer of the multiple oligonucleotide primer complex, and (C) a third sequence that is complementary to the third primer of the multiple oligonucleotide primer complex;

(ii) the second primer of the multiple oligonucleotide primer complex comprises (A) a sequence that is complementary to the second sequence of the first primer of the multiple oligonucleotide primer complex and (B) a signal generating moiety selected from the group consisting of a fluorescent agent and a chemiluminescent agent;

(iii) the third primer of the multiple oligonucleotide primer complex comprises (A) a sequence that is complementary to the third sequence of the first primer of the multiple oligonucleotide primer complex and ~~(b)~~ (B) a moiety capable of quenching, masking or inhibiting the activity of the signal generating moiety when located adjacent to, or in close proximity to the signal generating moiety; and

(iv) when the first, second and third primers of the multiple oligonucleotide primer complex are bound to one another, a signal generated by the signal generating moiety is inhibited;

(c) adding at least one reverse primer comprising a sequence that is substantially identical to a portion of the circular oligonucleotide probe;

(d) adding a DNA polymerase having strand displacement activity and lacking 3' to 5' exonuclease activity; and

(e) amplifying the circular oligonucleotide probe using ramification-extension amplification method (RAM) thus producing an amplification product comprising a sequence that is substantially identical to a sequence in the circular oligonucleotide probe, and separating the signal generating moiety from the quenching, masking or inhibitory moiety to generate a signal by the action of the DNA polymerase having strand displacement activity and lacking 3' to 5' exonuclease activity during the amplification method, wherein detection of the signal indicates the presence of the target nucleic acid in the nucleic acid containing sample.

25. – 32. (Cancelled)

33. (Currently Amended) A method for detecting a target nucleic acid in a target nucleic acid in a nucleic acid containing sample comprising:

(a) contacting the nucleic acid containing sample with a circular oligonucleotide probe under conditions that allow hybridization between complementary sequences in the target nucleic acid and the circular oligonucleotide probe;

(b) adding at least one forward primer comprising a sequence that is complementary to a portion of the circular oligonucleotide probe, under conditions where the forward primer is extended around the circular oligonucleotide probe for multiple revolutions to form a single-stranded DNA molecule comprising repeating units complementary to the sequence of the circular oligonucleotide probe;

(c) adding at least one multiple oligonucleotide primer complex comprising a first primer, a second primer and a third primer, wherein

- (i) the first primer of the multiple oligonucleotide primer complex comprises (A) a first sequence on its 3' end that is substantially identical to a portion of the circular oligonucleotide probe, (B) a second sequence that is complementary to the second primer of the pair, and (C) a third sequence that is complementary to the third primer of the multiple oligonucleotide primer complex;
 - (ii) the second primer of the multiple oligonucleotide primer complex comprises (A) a sequence that is complementary to the second sequence of the first primer of the multiple oligonucleotide primer complex and (B) a signal generating moiety selected from the group consisting of a fluorescent agent and a chemiluminescent agent;
 - (iii) the third primer of the multiple oligonucleotide primer complex comprises (A) a sequence that is complementary to the third sequence of the first primer of the multiple oligonucleotide primer complex and ~~(B)~~ (B) a moiety capable of quenching, masking or inhibiting the activity of the signal generating moiety when located adjacent to, or in close proximity to the signal generating moiety; and
 - (iv) when the first, second and third primers of the multiple oligonucleotide primer complex are bound to one another, a signal generated by the signal generating moiety is inhibited; and
- (d) adding a DNA polymerase having strand displacement activity and lacking 3' to 5' exonuclease activity; and

(e) amplifying the circular oligonucleotide probe using ramification-extension amplification method (RAM) thus producing an amplification product comprising a sequence that is substantially identical to a sequence in the circular oligonucleotide probe, and separating the signal generating moiety from the quenching, masking or inhibitory moiety to generate a signal by the action of the DNA polymerase having strand displacement activity and lacking 3' and 5' exonuclease activity during the amplification method, wherein detection of the signal indicates the presence of the target nucleic acid in the nucleic acid containing sample.

34. (Currently Amended) A method for detecting a target nucleic acid in a nucleic acid containing sample comprising:

(a) contacting the target nucleic acid containing sample with a linear oligonucleotide probe comprising 3' and 5' regions complementary to adjacent sequences in the target nucleic acid under conditions that allow hybridization between complementary sequences in the target nucleic acid and the linear oligonucleotide probe, whereupon binding to the target nucleic acid, the 3' and 5' ends of the linear oligonucleotide probe are adjacent to each other such that ligation of the 3' and 5' ends of the linear oligonucleotide probe forms the circular oligonucleotide probe, ligating the 3' and 5' ends of the linear oligonucleotide probe, and forming a circular oligonucleotide probe when the target nucleic acid is in the nucleic acid containing sample;

(b) adding at least one forward primer comprising a sequence that is complementary to a portion of the circular oligonucleotide probe, under conditions where the forward primer is extended around the circular oligonucleotide probe for multiple revolutions to form a

single-stranded DNA molecule comprising repeating units complementary to the sequence of the circular oligonucleotide probe;

(c) adding at least one multiple oligonucleotide primer complex comprising a first primer, a second primer and a third primer, wherein

(i) the first primer of the multiple oligonucleotide primer complex comprises (A) a first sequence on its 3' end that is substantially identical to a portion of the circular oligonucleotide probe, (B) a second sequence that is complementary to the second primer of the pair, and (C) a third sequence that is complementary to the third primer of the multiple oligonucleotide primer complex;

(ii) the second primer of the multiple oligonucleotide primer complex comprises (A) a sequence that is complementary to the second sequence of the first primer of the multiple oligonucleotide primer complex and (B) a signal generating moiety selected from the group consisting of a fluorescent agent and a chemiluminescent agent;

(iii) the third primer of the multiple oligonucleotide primer complex comprises (A) a sequence that is complementary to the third sequence of the first primer of the multiple oligonucleotide primer complex and ~~(b)~~ (B) a moiety capable of quenching, masking or inhibiting the activity of the signal generating moiety when located adjacent to, or in close proximity to the signal generating moiety; and

(iv) when the first, second and third primers of the multiple oligonucleotide primer complex are bound to one another, a signal generated by the signal generating moiety is inhibited; and

(d) adding a DNA polymerase having strand displacement activity and lacking 3' to 5' exonuclease activity; and

(e) amplifying the circular oligonucleotide probe using ramification-extension amplification method (RAM) thus producing an amplification product comprising a sequence that is substantially identical to a sequence in the circular oligonucleotide probe, and separating the signal generating moiety from the quenching, masking or inhibitory moiety to generate a signal by the action of the DNA polymerase having strand displacement activity and lacking 3' and 5' exonuclease activity during the amplification method, wherein detection of the signal indicates the presence of the target nucleic acid in the nucleic acid containing sample.

35. – 55. (Cancelled)